Developmental Cell
Previews

Pushing Single-Gene Genetic Analysis up a Notch

Ernst Hafen[1,*]
[1]Institute of Molecular Systems Biology, ETH Zürich, 8093 Zürich, Switzerland
*Correspondence: ehafen@ethz.ch
DOI 10.1016/j.devcel.2009.05.006

Genetic screens in *Drosophila* have come a long way since the pioneering work of Nüsslein-Volhard and Wieschaus. A recent paper by Mummery-Widmer et al. illustrates the use of genome-wide RNAi-based reverse genetic screens in vivo and systems biology approaches to identify new components of the Notch signaling pathway.

In his undergraduate lecture on introductory biology at MIT, Eric Lander introduces the two fundamentally different approaches to studying biology, genetics and biochemistry, in the following way: the geneticist studies an organism minus one gene. The biochemist studies a protein minus an organism. Some 40 years ago, molecular biology and gene cloning connected mutated genes and encoded proteins and provided a basis for identifying the fundamental mechanisms of cell and developmental biology. The latest and technologically most advanced tour de force genetic screen directed at understanding Notch signaling during development has just been published in Nature (Mummery-Widmer et al., 2009). As well as being impressive in its own right, this study provides a prompt to review the advantages and disadvantages of the different genetic screens used over time, and ask what else is needed to take us from describing basic biological mechanisms to understanding how the genome of an individual species specifies its phenotype (e.g., its precise size and shape).

Our understanding of the mechanisms underlying the development of multicellular organisms started to grow exponentially with the pioneering *Drosophila* embryonic lethal genetic screen carried out by Christiane Nüsslein-Volhard and Eric Wieschaus almost 30 years ago (Nüsslein-Volhard and Wieschaus, 1980). At the time, this approach was greeted with great skepticism by the *Drosophila* community, and "mindless" screening was also regarded by many as not really being intellectual science. Together with the work on homeotic genes by Edward Lewis, Nüsslein-Volhard and Wieschaus proved the skeptics wrong and provided a foundation for understanding how the body plan of insects and vertebrates is laid out. Since then, whole-genome screens have been conducted in almost endless variations and have contributed to our present-day understanding of fundamental biological mechanisms. The discovery of RNA interference (RNAi) has opened up genetic dissection of biological processes in organisms less genetically tractable than *Drosophila* and *C. elegans*. At this point, the "organism-minus-one-gene" approach can be used in mammalian cells, in tissues, and in entire organisms. Even in *Drosophila*, the availability of a genome-wide collection of transgenic inducible RNAi lines has provided a new dimension in genetic resolution. In their new paper, Mummery-Widmer et al. (2009) demonstrated the power of this new approach by applying it to the Notch pathway.

To expand our understanding of Notch signaling, the authors chose to use external sensory organs, the bristles on the back of the fly, to screen for Notch signaling components. It is known that Notch acts twice during the development of each bristle organ—first, to ensure that from each group of putative sensory organ precursor cells (SOP), only one is selected as an SOP cell, and second, to ensure that during the two asymmetric divisions of the SOP, the four cells of the bristle organ (socket, shaft, neuron, and sheath cell) are correctly specified. Based on the phenotypes of known Notch pathway mutants, they classified the phenotypes of lines with RNA hairpin expression induced in the region where bristle organs develop into basic phenotypic classes: defects in asymmetric cell division (226 genes) and lateral inhibition (233 genes). For 201 lines in this latter class, RNA hairpin expression was also induced in the wing as a test for general Notch regulation, which narrowed the pool down to 23 genes not previously implicated in Notch signaling. Using a reporter line for Notch target genes, these 23 regulators were further classified according to whether they act upstream of Suppressor of Hairless, the key transcription factor in the Notch signaling pathway. Similarly, reporter lines were used to determine whether "loss of bristle" phenotypes were caused by the failure to select SOPs or by defects in asymmetric division. With this detailed phenotypic and genetic characterization in hand, the authors then integrated existing interaction data from yeast two-hybrid screens, biochemistry, and genetics using MCODE (Bader and Hogue, 2003) to construct a Notch signaling network of 177 genes with 780 interactions. Many of the highly interconnected nodes represent known protein complexes involved in Notch modification, signaling, and transcriptional output. The validity of these complexes was confirmed by functional testing of additional members. This unprecedented resolution of Notch signaling in vivo also highlights the role of components involved in vesicle transport and in ubiquitinylation of Notch and other signaling components.

The paper by Mummery-Widmer and colleagues is an impressive demonstration of the power of the whole-genome, tissue- or cell-type-specific RNAi screens that are now possible in *Drosophila*. One of the main advantages of this type of reverse genetic screen relative to classical forward genetic screens is that the targeted gene is already known, which eliminates the need to map the mutation. It is also more straightforward to assay multiple phenotypes at once, as one can always go back to the same gene or its RNAi line. For a more detailed characterization of different genetic screening methods, see Table 1.

Table 1. Comparison of Different Genetic Screening Methods

Type of Screen	Mutagen/Effect	Mapping	Advantages	Disadvantages
EMS screen for recessive mutations (Nüsslein-Volhard and Wieschaus, 1980)	chemical (EMS)	recombination mapping and complementation analysis	• allelic series • point mutations identify functional domains in protein	• F3 screen • mapping tedious • lines selected for a specific phenotype • lethality precludes analysis of later phenotypes
Tissue-specific EMS screen using eye-Flp (Newsome et al., 2000; Gluderer et al., 2008)	chemical (EMS)	recombination mapping and complementation analysis	• F1 screen (>10-fold genome saturation possible) • genetic mosaics circumvent problems with organismal lethality • allelic series • point mutations identify functional domains in protein • slow (e.g. 10 person years for 10-fold saturation of genome for growth mutants)	• mapping tedious • lines selected for a specific phenotype
Tissue-specfic RNAi screen (Dietzl et al., 2007)	RNA interference	no mapping necessary	• F1 screen • no mapping required • forward screen permits recording multiple phenotypes • targeted gene knockdown controlled by Gal4 line (also possible in postmitotic cells) • fast (6 person years for entire screen and characterization of mutants)	• gene inactivation often not complete • no allelic series • no information about functional domains of the protein • activation of the RNA interference response • off-target effects

At this point, the technology and resources for similar RNAi-based screens in other organisms, including *C. elegans* and the mouse, are simply not available. It is likely that many of the 23 new gene products that play a role in Notch signaling are conserved during evolution and that they play a similar role in vertebrates. But what have we learnt beyond that? Can we now predict or model how the Notch switch between two cell fates is thrown? Even this sophisticated organism-minus-one-gene approach has only given us a resolution of minus 600 million years, the time of the last common ancestor between vertebrates and invertebrates. We still need to understand how the activity of these conserved pathways (e.g., Notch) controls the different sizes and shapes of species and the variation within species. To attain this important goal, we have to move away from the single-gene approach and study the interaction between these conserved gene and signaling networks in a quantitative way. One of the promises of systems biology is that emergent properties within these networks will help us to understand fundamental differences between biological systems. To attain this next level of understanding, not only do we need quantitative data sets of RNA and protein expression and modification in time and space, we will also need computational tools to integrate and model these interactions. Such computational models must also include the physical properties of cells and tissues, and must make predictions that can be tested experimentally with tools such as an RNAi library that enables simultaneous knockdown of multiple genes.

Because natural and artificial selection acts on cellular networks and not on individual genes, complementary information will come from population genetics. The rapidly decreasing cost of DNA sequencing permits the accumulation of large numbers of DNA sequences. With the "$1000 genome" being only a few years away, this even applies to humans. Unlike model organisms, humans will be able to contribute detailed phenotypic information, permitting a high-resolution mapping of phenotype to genotype. Taking this approach together with the detailed understanding from the single-gene genetic approaches in model organisms, we can be optimistic about understanding and modeling biological processes and diseases in more quantitative and complete ways in the future.

REFERENCES

Bader, G.D., and Hogue, C.W. (2003). B.M.C. Bioinformatics *4*, 2.

Dietzl, G., Chen, D., Schnorrer, F., Su, K.C., Barinova, Y., Fellner, M., Gasser, B., Kinsey, K., Oppel, S., Scheiblauer, S., et al. (2007). Nature *448*, 151–156.

Gluderer, S., Oldham, S., Rintelen, F., Sulzer, A., Schütt, C., Wu, X., Raftery, L.A., Hafen, E., and Stocker, H. (2008). BMC Dev. Biol. *8*, 10.

Mummery-Widmer, J.L., Yamazaki, M., Stoeger, T., Novatchkova, M., Bhalerao, S., Chen, D., Dietzl, G., Dickson, B.J., and Knoblich, J.A. (2009). Nature *458*, 987–992.

Newsome, T.P., Asling, B., and Dickson, B.J. (2000). Development *127*, 851–860.

Nüsslein-Volhard, C., and Wieschaus, E. (1980). Nature *287*, 795–801.